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METHODS IN PLANKTOLOGY.[1]

GEORGE WILTON FIELD.

HUMAN existence is dependent upon the oceanic fauna and flora far more than is generally suspected. Scientific investigation has demonstrated a most remarkable biological chain and has elucidated the links which connect the lowliest of the microscopic plants with the most highly developed mammals. In the continual cycle of matter from inorganic to organic, from organic to inorganic, with the attendant alternate storing up and liberation of energy, are to be found the secrets at the basis of life. It is commonly held by biologists that life originated in the sea ; and it is in the sea to-day that we find those plants and animals which have departed least from the original, the ancestral condition, in which life is not complicated by diversity of form or function.

Some of the work carried on by the biological department of the Rhode Island Experiment Station has been upon the Methods of Studying the Œcology of Marine Organisms, since a knowledge of the marine organism is of immense importance in understanding the questions connected with the fundamental food supply on the earth.

The number and variety of the animal and vegetable population of the ocean are well-nigh infinite. Any two regions more or less remote from each other show differences in their oceanic fauna and flora, generally proportional to the distance either horizontal or vertical which separates them. The fauna and flora of the tropical Caribbean Sea differs widely from that of the Arctic oceans ; that of the water south of Cape Cod differs markedly from that north of the Cape, though separated only by a very few miles of land. The organisms characteristic of the surface in any region are wonderfully different from those of the abyssal depths. Yet even in the same locality remarkable variations are the rule. These variations are conditioned not only by temperature, specific gravity, atmospheric pressure, and. light, but probably by fundamental phenomena of which science as yet knows nothing. Certain forms spend the day in the depths, appearing at the surface only at night ; for various forms the reverse is true. There are other temporal differences, — yearly, monthly, daily, and hourly variations, — whose causes are manifold, in part climatic or meteorological, in part depending upon the conditions of life, of reproduction, and development. Still other variations are brought about by the numberless currents great and small, which not only collect the organisms into eddies and scatter the "schools," but transport organisms characteristic of one region to places far remote from their home, *e.g.*, the Gulf Stream carries tropical forms far into the cold northern seas.

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[1] Reprinted from the *Annual Report of the R. I. Agricultural Experiment Station*, 1897.

All the organisms which are borne about helplessly by currents, or whose motions are determined by protoplasmic activities (heliotropism, chemiotropism, etc.), as distinguished from special and effective locomotory organs, constitute the Plankton (a word coined by Professor Hensen from the Greek *πλανᾶσθαι*, to wander). The Plankton has attracted naturalists since the studies of Johannes Müller, but Professor Hensen was the first to give earnest attention to the economic importance of the Plankton, and to the problems of the food supply based upon it. He was lèd to this through his attempt to get an approximate idea of the number of fish in corresponding districts. This work brought him to the question of the food supply for these fishes, and from that to consideration of the general primary sources of food and the cycle of matter in the ocean. This has led to important results in tracing the cycle of changes through which the organic elements, carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, iron, and others pass ; in showing how they either singly, or united in simple combinations, become incorporated into a living (it may be microscopic) plant ; how this plant is eaten by a mollusc or a small fish, a prey in turn for larger and fiercer fish, which ultimately die and are broken up by microscopic plants (bacteria) into the original elements, to again nourish plants. The actual cycle is rarely so simple as described above. Complicating conditions usually appear at every stage. Naturalists are gradually unraveling these complications. But the point which is of special importance is that very many of these marine animals may furnish economical, healthful, and delicious food for man. That this may be a never-failing source of food supply for an increasing human population, not only must the habits, haunts, and life histories of such food animals (fishes, molluscs, crustacea, etc.) be elucidated, but also their relation to natural phenomena, meteorological conditions, currents, etc., and especially to the Plankton, upon which they depend more or less immediately for food. This necessitates study of the Plankton as the basis of food supply for our most important marine food animals.

The study of the economic aspects of the Plankton and the application of the results to cultivation of water areas have demonstrated that the water responds even more bountifully than land areas to cultivation. It is an interesting economic fact that less than 15 cubic feet of cultivated water is sufficient to support at least the head of a family (and probably a considerable number of other dependents) of Italians in Tarente, while 6 cubic feet do the same in Japan. Numerous experiments demonstrate that the yield of cultivated water area surpasses in essential food elements that of equal area of cultivated land. Herein lies the great importance of a knowledge of the Plankton, the basis of marine life. The Plankton also enters as an important and, in certain aspects, as an undesirable element into the question of municipal water supplies, and the necessity of healthful and palatable drinking water has stimulated not a little the study of the quantitative and qualitative constitution of the Plankton.

Since the time (1884) when Hensen entered upon his work of counting laboriously the number of organisms in known quantities of sea water, for the purpose of ascertaining the amount of living matter which exists in given volumes of water, and thus furnishing a basis for scientific aquaculture, much attention has been given to the methods of Planktology and rapid progress has been made. The great desideratum even now is a rapid, simple method by which data can be obtained which can be used for comparison of all waters. Not until the invention of such a method can accurate and valuable comparisons be made.

At the basis lies the method of collecting the organisms from an accurately determined quantity of water. An ideal method is one which includes the concentration of the organic matter in a known quantity of water into a smaller known quantity of water, which quantity should be a convenient multiple of the original quantity. In the process not even the smallest of the bacteria should be lost. Counting and enumeration of individuals and species is necessary, together with an estimation as accurate as possible of the volume of the water, of the inorganic matter, and of the organic amorphous débris (plant and animal). The counting can best be done by the Sedgwick-Rafter method (Rafter, G. W., '92). By this method a fairly accurate idea can be formed of the comparative volumetric and numerical proportions between the three main elements involved in the biological study of water; *viz.*, the living organisms, organic amorphous débris, and inorganic substances (silt, gases, and substances in solution). It would seem that the necessary data must be based ultimately upon the counting method until such time as means can be devised for separating the living organic from the dead (both organic and inorganic) substance, and for determining the amount of each. In considering the quantity of living organisms not only the number but also the size of the individuals must be taken into account. Professor Hensen introduced the counting methods for the purpose of determining the economic yield of the ocean in the same way as the farmer determines the useful yield of his fields and meadows, the annual production of grass and grain. Professor Haeckel in stating his objections to this method said: "The farmer determines the yield of his meadows, garden, and field by quantity and weight, not by counting the individuals. If instead of this he wished to introduce Hensen's new exact method of determination, he must count all the potatoes, kernels of grain, grapes, cherries, etc., not only that but he must also count the blades of grass in his plot, even every individual weed which grows among the grain of his field and the useful plants of his garden, for these also, regarded from the physiological point of view, belong to the 'total production' of the ground." (Translation in Report of U. S. Commissioner of Fish and Fisheries for 1889–91, pp. 565–641, of *Plankton Studien, Jenaische Zeitschrift,* Bd. xxv, 1890.) It would seem as if Professor Haeckel overlooked the fact that the farmer can readily separate the hay, etc., from stones, dead sticks, and other foreign material. He can accurately determine the volume and weight of farm products. He does not have to contend in this connection with foreign substances, such as silt, organic débris, etc., which render inaccurate determinations by weight and volume of the contents of water from ponds, lakes, and oceans. It is these elements which thus far have prevented any apparent progress in establishing tables of the economic yield of water volumes on the basis of weight, volume, and number of individuals, which would be of value for comparison in determining the commercial importance of any area or any depth of water.

It is, too, the presence of an undetermined and locally varying quantity of organic débris which renders inaccurate the estimation of the economic value of water by means of the determination of the albuminoid ammonia.

Numerous methods of Plankton collecting have been devised; the most important of these may be grouped as follows:

(1) By drawing a fine net through known volumes of water.

(2) By passing known volumes of water through a filter of either (*a*) fine silk bolting cloth, or (*b*) fine sand, or (*c*) a combination of *a* and *b*.

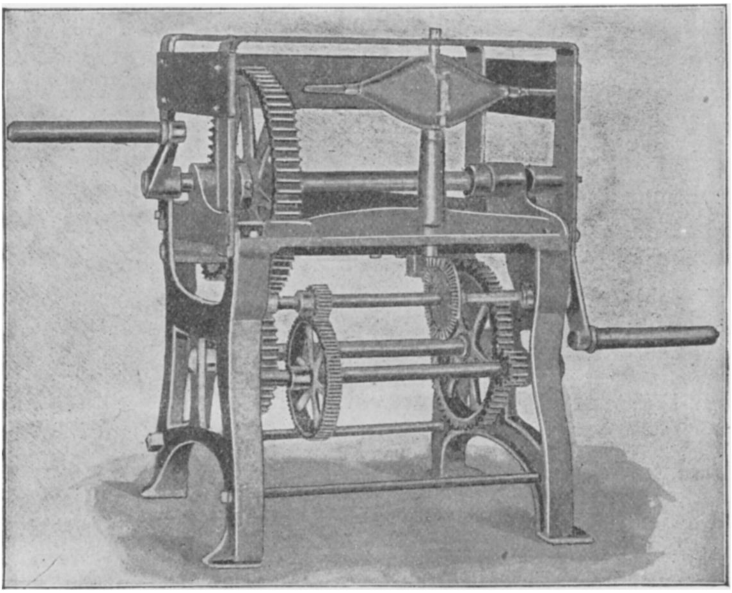
(1) The net and the method of using it have been subjected to much study by Hensen ('**87** and '**95**), Apstein ('**91**, '**92**, and '**96**), Reighard ('**94**), Ward ('**96** and '**96a**), Borgert ('**96**), Kofoid ('**97**), and others. From the net method it seems impossible to exclude several prolific sources of uncertainty in the results; *viz.,* (*a*) it is impossible to be *certain* of the quantity of water through which the net is drawn, and consequently of the quantity which passes through the net even in motionless water; (*b*) currents in the water almost hopelessly complicate the conditions; (*c*) the progressive clogging of the net cannot be

avoided ; (*d*) there is an actual loss of small individuals through the meshes of the net ; (*e*) the long and complicated process must necessarily give varied results due to personal variations in methods of work, and to changes in the local conditions, *e.g.*, the rate of currents may vary from day to day, the quantity of silt may modify the filtering capacity of the net, etc. Any one of these sources of error is sufficient to invalidate the entire method, rendering the results worthless for comparison with the results of similar processes in different localities.

Under (2) (passing known quantities of water through a filter of fine bolting cloth) the sources of error are reduced but not eliminated ; (*a*) the pressure of water forces certain small forms, *e.g.*, certain species of bacteria, through the meshes. Many of the very delicate forms may be broken up and destroyed ; (*b*) failure to wash out all the individuals from the net. The method of pumping known volumes employed by Kofoid ('97) is particularly good. The most apparent source of error is the control of the quantity of water pumped, and the possibility that the strong suction of the pump used may draw mud when the water is taken within a foot of the bottom. In the method of filtration through sand, as employed by Calkins ('91) and as improved by Jackson ('96) and by Whipple ('96), the possible sources of error are several, varying with the characteristics of the sand used, with the shape of the funnel, and with the nature of the organic matter in the water. Calkins says : "The sloping sides of the glass funnel offer a surface for the settling of organisms, and the error arising in this way may be considerable. A water free from amorphous matter and zoogl∞a will filter very accurately, but a water containing these gives opportunity for error." Jackson ('96) adds : "This is undoubtedly due to the jelly-like character of the zoogl∞a, and to the fact that while adhering to the funnel sides itself, it also retains with it other organisms." . . . "Not only do amorphous matter and zoogl∞a readily adhere to the sides of the ordinary glass filter funnel, but the same is true of the gelatinous growths of the Cyanophyceæ and of the flocculent threads of Crenothrix." Even Jackson's ('96) improvements in the sand filtration method which reduce to a minimum the liability of error cannot remove the defects inherent in the process itself. The defects noted by Whipple ('96) are involved in the method of concentrating the sample, *viz.*—(1) the funnel error, arising from the adherence of organisms and amorphous débris to the sides of the funnel ; (2) the sand error, caused by organisms passing through the sand ; (3) the decantation error, resulting from the adhesion of organic matter to the particles of sand, and from the capillary retention in the sand of the water used in washing the sand during decantation ; to the above should be added (4) the destruction of the very delicate organisms by the sand in the process of decantation. The practical value of the method for comparative results in the hands of different workers is invalidated by the multiplicity of conditions affecting the results; among these are the nature and amount of the sand, the care and skill of the worker, and particularly the nature of the sample to be filtered.

Kofoid (*Science*, vi, 153, Dec. 3, 1897, "On Some Important Sources of Error in the Plankton Method") found that filter paper (No. 575 Schleicher & Schüll) was more effective than the sand filtration method, giving 75% to 85% of the planktonts as compared with 40% to 65% given by the sand filters. Kofoid has detected the advantage of filtration through very delicate porous media, and finds that fine infusorial earth is very efficient, and in spite of minor difficulties connected with the final separation of the planktonts from the infusorial earth he regards this as the most satisfactory method thus far devised. I might add that the total weight of material (organisms, organic and inorganic débris) suspended in water is of fundamental importance and can be determined with considerable accuracy by this method, though I see no way to ascertain the relative proportion of organisms and débris except very roughly through the enumeration of the individual organisms and comparison of the apparent bulk of the masses of living and dead material as seen under the microscope.

Experiments have been made by adding various quantities of either corrosive sublimate, picric acid, acetic, and other acids, alcohol, and formalin to known quantities of water, with a subsequent determination of the volume and constituent elements of the precipitate. The space required, the tediousness, the loss of organisms, the fact that in this new process very many forms break up before all the material is settled have led to its abandonment in favor of the employment of centrifugal force. Previous to 1896 Cori devised a simple hand centrifuge and used it for collecting infusoria for class work. Last year a brief reference was made to our work with the centrifugal method (Field, '97, I). Since then Kofoid ('97) has experimented on similar lines. His machine is "geared to give 3000 to 4000 revolutions per minute and arranged to act upon a continuous stream of water, all of which was subjected to the maximum and uniform action of the centrifugal force." This machine secured in some instances 98% of the planktonts. But as I pointed out last year, it is not so efficient with those organisms whose specific gravity is about that of water, such as the Cyanophyceæ, Anabœna, Clathrocystis, *et al.*



The Planktonokrit.

Experiments have been made with the centrifugal machine devised by Dr. C. S. Dolley, called the Planktonokrit, and described by him (Dolley, '96) : "An apparatus which consists of a series of geared wheels driven by hand or belt, and so arranged as to cause an upright shaft to revolve to a speed of 8000 revolutions per minute, corresponding to 50 revolutions per minute of the crank or pulley wheel. To this upright shaft is fastened an attachment by means of which two funnel-shaped receptacles of one liter capacity each, may be secured and made to revolve with the shaft. The main portion of each of these receptacles is constructed of spun copper, tinned. To this is attached the stem of the funnel, consisting of a heavy annealed glass tube of 15 mm. in outside diameter with a central bore of 2½ to 5 mm. These glasses are held in place and protected by a cover, such as is employed in mounting a water gauge.

"The receptacle having been filled with the water to be examined, is caused to revolve for one or two minutes, when the entire contents of suspended matter in the water is thrown down to the bottom of the tube, from which the volume may be read off by means of the graduated scale on the outside of the tube. The plankton thus expeditiously secured can be transferred quickly to a vial or other receptacle, to be weighed or otherwise examined at leisure."

Power may be applied either by hand or through a belt by steam or electric motor.

Our experience with the Planktonokrit indicates :

(1) That two men on each crank cannot get a speed at the receptacle above 3000 revolutions per minute; a rate, however, sufficient to throw out everything except the Cyanophyceæ.

(2) The maximum speed must be continued for at least four minutes.

(3) A speed much above 4000 revolutions with such a large quantity of water is dangerous, with the machine constructed as at present.

This danger may be obviated :

(*a*) By reducing the capacity of the receptacle. (500 cc. is probably sufficient.)

(*b*) By lengthening the bearings of the upright spindle.

(*c*) By enclosing the revolving receptacles in a circular chamber, thus lessening the resistance of the air.

(4) When power was used, more satisfactory results were obtained by arranging the driving pulley so as to cut out the two largest sets of gears. Friction was thereby greatly reduced, and the necessary speed was gained from more rapid revolution of the driving pulley.

(5) It is probable that four receptacles would work more satisfactorily than two.

Some difficulty was experienced in avoiding leaks at the ends of the glass tube. At the distal end the insertion of a closely fitting, vaselined rubber "mushroom" (such as is used when repairing punctures in bicycle tires) was found to answer. Care had to be exercised to keep the entrance to the tube free at the proximal end. To obviate this difficulty it is hoped that a small, heavy annealed glass cone with a ground glass stopper at the apex can be devised in place of the tube.

That the centrifugal method is beyond question the best method of collecting the substances suspended in the water for accurate determination seems to be proved, and great credit is due to Dr. Dolley for his demonstration of the fact.

This method is of value, not alone to him who wishes to determine the proportions of organic matter in drinking water, and to ascertain the quantity of microscopic plants and animals in water from special localities (a very accurate index of its commercial value for fish and shellfish cultivation), but it will enable biologists to study more successfully those lowly forms which lie close to the basis of life, the delicacy of whose structure precludes handling by nets or filters.

It is believed that the perfecting of the centrifugal method for collecting the Plankton will greatly facilitate the practical solution of the increasingly important question of the food supply for man, by ameliorating some of the difficulties which surround the rearing of edible fish in confinement. The eggs can be hatched by millions, but difficulty arises in obtaining a natural or proper food supply. Hence in the case of most species the fry must be liberated very soon after hatching. But every additional day in which they can be kept in confinement increases in a remarkably large ratio the number of these young fish which attain maturity, for the reason that the very young fry are specially liable to destruction from rapacious enemies, storms, etc. With the use of the centrifugal machines for collecting the microscopic food for the young fry, they can be kept longer in confinement, and probably the advantage may be twofold, for in addition to diminishing the mortality, we should expect that growth would be accelerated under the influence of abundant food.

The Rhode Island Agricultural Experiment Station maintains a card catalogue of works upon the subjects connected with Investigations on the Plankton. Printed copies of the Bibliography will be sent upon application. Workers are requested to forward reprints of their papers. Address Biological Division, R. I. Experiment Station, Kingston, R. I.